Supporting Information

Discovery of New 1,1'-Biphenyl-4-sulfonamides as Selective Sub-nanomolar Human Carbonic Anhydrase II Inhibitors

Giuseppe La Regina, Michela Puxeddu, Marianna Nalli, Daniela Vullo, Paola Gratteri, Claudiu Trandafir Supuran, Alessio Nocentini,* and Romano Silvestri*

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Chemistry. All reagents and solvents were handled according to the material safety data sheet of the supplier and were used as purchased without further purification. MW-assisted reactions were performed on a CEM Discover SP single mode reactor, controlling the instrument settings with PC-running CEM Synergy 1.49 software. Closed vessel experiments were carried out in dedicated capped vials (10 mL) with cylindrical stirring bar (length 8 mm, diameter 3 mm). Stirring, temperature, irradiation power, maximum pressure (Pmax), PowerMAX (simultaneous cooling-while-heating), ActiVent (simultaneous venting-while-heating), and ramp and hold times were set as indicated. Temperature of the reaction was monitored by an external fiber optic temperature sensor. After completion of the reaction, the mixture was cooled to 25 °C via air-jet cooling. Organic solutions were dried over anhydrous sodium sulfate. Evaporation of solvents was carried out on a Büchi Rotavapor R-210 equipped with a Büchi V-850 vacuum controller and a Büchi V-700 vacuum pump. Column chromatography was performed on columns packed with silica gel from Merck (70−230 mesh). Silica gel thin layer chromatography (TLC) cards from Merck (silica gel precoated aluminium cards with fluorescent indicator viewable at 254 nm) were used for TLC. Developed plates were visualized with a Spectroline ENF 260C/FE UV apparatus. Melting points (mp) were determined on a Stuart Scientific SMP1 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a PerkinElmer Spectrum 100 FT-IR spectrophotometer equipped with a universal attenuated total reflectance accessory. IR data acquired and processed by PerkinElmer Spectrum 10.03.00.0069 software. Band position and absorption ranges are given in cm^{-1}. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded with a Varian Mercury (300 MHz) or a Bruker Avance (400 MHz) spectrometer in the indicated solvent, and the corresponding fid files were processed by MestreLab Research SL MestreReNova 6.2.1-769 software. Chemical shifts are expressed in δ units (ppm) from tetramethylsilane. Mass spectra were recorded on a Bruker Daltonics MicroTOF LC/MS mass spectrometer equipped with a positive ion ESI source. The purity of tested compounds was checked by high pressure liquid chromatography (HPLC) and it was found to be >95%. Thermo Fisher Scientific Inc. Dionex UltiMate 3000 HPLC system consisted of an SR-3000 solvent rack, a LPG-3400SD quaternary analytical pump, a TCC-3000SD column compartment, a DAD-3000 diode array detector, and an analytical manual injection valve with a 20 μL loop. Samples were dissolved in acetonitrile (1 mg/mL). HPLC analysis was performed by using a Thermo Fisher Scientific Inc. Acclaim 120 C18 column (5 □m, 4.6 mm × 250 mm), at 25 ± 1 °C with an appropriate solvent gradient (acetonitrile/water), flow rate of 1.0 mL/min and signal detector at 206, 230, 254 and 365 nm. Chromatographic data were

acquired and processed by Thermo Fisher Scientific Inc. Chromeleon 6.80 SR15 Build 4656 software.

General Procedure for the Preparation of Derivatives 1-3. Example: 4'-(2"-aminobenzoyl)-1,1'-biphenyl-4-sulfonamide (1). A mixture of **22** (60 mg, 0.16 mmol), $SnCl_2 \cdot H_2O$ (176 mg, 0.78 mmol) in ethyl acetate (5 mL) was refluxed for 3 h. After cooling, the reaction mixture was made alkaline with a solution of $NaHCO_3$, extracted with ethyl acetate and dried. Removal of the solvent gave **1** (36 mg, 64%), mp 62-65 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 6.52 (t, *J* = 4.4 Hz, 1H), 6.86 (d, *J* = 5.6 Hz, 1H), 7.13 (s, 2H, disappeared on treatment with D_2O), 7.29-7.33 (m, 2H), 7.43 (br s, 2H, disappeared on treatment with D_2O), 7.67 (d, *J* = 7.9 Hz, 2H), 7.87 (d, *J* = 7.2 Hz, 2H), 7.92-7.94 ppm (m, 4H). IR: ν 1648, 3261 cm^{-1}. $C_{19}H_{16}N_2O_3S$. MW 352.41. LRMS found (ESI) 353.3 (MH^+).

4'-(3"-Aminobenzoyl)-1,1'-biphenyl-4-sulfonamide (2). Synthesized as **1** starting from **23**. Yield 32%, mp 208-210 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 5.41 (s, 2H, disappeared on treatment with D_2O), 6.85 (t, *J* = 7.6 Hz, 2H), 6.97 (s, 1H), 7.19, (t, *J* = 6.9 Hz, 1H), 7.44 (br s, 2H, disappeared on treatment with D_2O), 7.82 (d, *J* = 8.5 Hz, 2H), 7.90-7.95 ppm (m, 6H). IR: ν 1646, 2918, 3259 cm^{-1}. $C_{19}H_{16}N_2O_3S$. MW 352.41. LRMS found (ESI) 353.1 (MH^+).

4'-(3"-Aminobenzyl)-1,1'-biphenyl-4-sulfonamide (3). Synthesized as **1** starting from **24**. Yield 22%, mp 182-185 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 3.79 (s, 2H), 4.94 (s, 2H, disappeared on treatment with D_2O), 6.35-6.39 (m, 3H), 6.90 (t, *J* = 7.8 Hz, 1H), 7.3 (d, *J* = 7.9 Hz, 2H), 7.35 (br s, 2H, disappeared on treatment with D_2O), 7.62 (d, *J* = 8.0 Hz, 2H), 7.79-7.86 ppm (m, 4H). IR: ν 3225 cm^{-1}. $C_{19}H_{18}N_2O_2S$. MW 338.42. LRMS found (ESI) 339.2 (MH^+).

General Procedure for the Preparation of Derivatives 4 and 5. Example: 4'-((2"-Aminophenyl)hydroxymethyl)-1,1'-biphenyl-4-sulfonamide (4). A mixture of **25** (100 mg, 0.28 mmol) and Pd/C (10 mg) in methanol:tetrahydrofuran 1:1 (3.5 mL) was hydrogenated under the pressure of 2 atm of hydrogen at room temperature for 4 h. The catalyst was removed by filtration and the obtained solution was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, ethyl acetate:*n*-hexane = 3:2 as eluent) to furnish **4** (20 mg, 20%) as an oil. ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 5.01 (s, 2H, disappeared on treatment with D_2O), 5.79 (d, *J* = 3.8 Hz, 1H), 5.95 (d, *J* = 4.2 Hz, 1H, disappeared on treatment with D_2O), 6.54 (t, *J* = 7.4 Hz, 1H), 6.60 (dd, *J* = 0.9 and 8.0 Hz, 1H), 6.96 (t. *J* = 7.9 Hz, 1H), 7.1 (dd, *J* = 1.3 and 7.6 Hz, 1H), 7.39 (br s, 2H, disappeared on treatment with D_2O), 7.50 (d, *J* = 8.2 Hz, 2H), 7.67 (d, *J* = 8.3 Hz, 2H), 7.83-7.89 ppm (m, 4H). IR: ν 2926, 3360 cm^{-1}. $C_{19}H_{18}N_2O_3S$. MW 354.42. LRMS found (ESI) 355.1 (MH^+).

4'-((3"-Aminophenyl)hydroxymethyl)-1,1'-biphenyl-4-sulfonamide (5). Synthesized as **4** starting from **26**. Yield 43%, mp 82-85 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 4.98 (s, 2H, disappeared on treatment with D_2O), 5.56 (d, *J* = 3.4 Hz, 1H), 5.75 (d, *J* = 3.7 Hz, 1H, disappeared on treatment with D_2O), 6.36-6.39 (m, 1H), 6.54 (d, *J* = 7.6 Hz, 1H), 6.58-6.60 (m, 1H), 6.92 (t, *J* = 7.9 Hz, 1H), 7.36 (br s, 2H, disappeared on treatment with D_2O), 7.45 (d, *J* = 8.0 Hz, 2H), 7.64 (d, *J* = 8.2 Hz, 2H), 7.80-7.87 ppm (m, 4H). IR: ν 3246 cm^{-1}. $C_{19}H_{18}N_2O_3S$. MW 354.42. LRMS found (ESI) 355.3 (MH^+).

1,1'-Biphenyl-4'-(3"-Oxo-1",3"-dihydroisobenzofuran-1-yl)-4-sulfonamide (6). A mixture of **35** (138 mg, 0.43 mmol), **28** (195 mg, 0.69 mmol) and potassium phosphate tribasic (274 mg, 1.3 mmol) in *N,N*-dimethylformamide (4 mL) was degassed for 30 min. $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (11 mg, 0.13 mmol) was added under Ar stream and the reaction mixture was heated at 60 °C for 2 h. After cooling, water and ethyl acetate were added and the

resulting mixture was filtered through a pad of silica gel. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, chloroform:ethyl acetate = 2:1 as eluent) to furnish **6** (20 mg, 13%), mp 236-239 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 6.81 (s, 1H), 7.39 (br s, 2H, disappeared on treatment with D_2O), 7.45 (d, *J* = 8.4 Hz, 2H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.65, (t, *J* = 7.8 Hz, 1H), 7.76-7.79 (m, 3H), 7.84-7.90 (m, 4H), 7.95 ppm (d, *J* = 7.2 Hz, 1H). IR: ν 1732, 3221, 3340 cm^{-1}. $C_{20}H_{15}NO_4S$. MW 365.40. LRMS found (ESI) 366.3 (MH^+).

General Procedure for the Preparation of Derivatives 7-11. Example: 2"-(1,1'-Biphenyl-4-sulfamoyl-4'-ylcarbonyl)benzoic acid (7). A mixture of **40** (50 mg, 0.13 mmol) and $LiOH{\cdot}H_2O$ (16 mg, 0.4 mmol) in tetrahydrofuran (2 mL) and water (2 mL) was stirred at room temperature overnight. The reaction mixture was made acid with 1N HCl and extracted with ethyl acetate; the organic layer was washed with brine, dried and filtered. Removal of the solvent gave **7** (40 mg, 77%), mp 220-223 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 7.43-7.45 (m, 3H, disappeared on treatment with D_2O), 7.64-7.75 (m, 4H), 7.8 (d, *J* = 8.2 Hz, 2H), 7.91 (s, 4H), 8.0 (d, *J* = 7.6 Hz, 1H), 13.22 ppm (br s, 1H, disappeared on treatment with D_2O). IR: ν 1665, 1686, 3333 cm^{-1}. $C_{20}H_{15}NO_5S$. MW 381.40. LRMS found (ESI) 382.3 (MH^+).

2"-(1,1'-Biphenyl-4-sulfamoyl-4'-ylmethyl)benzoic acid (8). Synthesized as **7** starting from **41**. Yield 95%, mp 218-220 °C (from ethanol). ^{1}H NMR 300 MHz (DMSO-*d*$_6$): δ 4.39 (s, 2H), 7.24-7.35 (m, 6H, disappeared on treatment with D_2O), 7.45-7.51 (m, 1H), 7.59-7.62 (m, 2H), 7.78-7.86 ppm (m, 5H). IR: ν 1686, 2925, 3263 cm^{-1}. $C_{20}H_{17}NO_4S$. MW 367.42. LRMS found (ESI) 368.2 (MH^+).

3"-(1,1'-Biphenyl-4-sulfamoyl-4'-ylcarbonyl)benzoic acid (9). Synthesized as **7** starting from **42**. Yield 85%, mp 249-251 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 7.44 (br s, 2H, disappeared on treatment with D_2O), 7.72 (t, *J* = 7.7 Hz, 1H), 7.87-7.89 (m, 2H), 7.93-8.04 (m, 7H), 8.23 (d, *J* = 7.7 Hz, 1H), 8.27 (s, 1H); 13.30 ppm (br s, 1H, disappeared on treatment with D_2O). IR: ν 1654, 1687, 2550, 3368 cm^{-1}. $C_{20}H_{15}NO_5S$. MW 381.40. LRMS found (ESI) 382.4 (MH^+).

3"-(1,1'-Biphenyl-4-sulfamoyl-4'-ylmethyl)benzoic acid (10). Synthesized as **7** starting from **43**. Yield 85%, mp 223-225 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 4.05 (s, 2H), 7.35 (br s, 2H, disappeared on treatment with D_2O), 7.37-7.43 (m, 3H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.75-7.86 ppm (m, 6H). IR: ν 1685, 2556, 2906, 3261 cm^{-1}. $C_{20}H_{17}NO_4S$. MW 367.42. LRMS found (ESI) 368.1 (MH^+).

3"-(1,1'-Biphenyl-4-sulfamoyl-4'-ylhydroxymethyl)benzoic acid (11). Synthesized as **7** starting from **44**. Yield 88%, mp 223-225 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 5.84 (s, 1H), 6.08 (br s, 1H, disappeared on treatment with D_2O), 7.17 (br s, 2H, disappeared on treatment with D_2O), 7.43 (t, *J* = 8.8 Hz, 1H), 7.5 (d, *J* = 7.6 Hz, 2H), 7.66-7.69 (m, 3H), 7.78-7.88 (m, 5H), 7.99 (s, 1H), 12.89 ppm (br s, 1H, disappeared on treatment with D_2O). IR: ν 1686, 2547, 3370 cm^{-1}. $C_{20}H_{17}NO_5S$. MW 383.42. LRMS found (ESI) 384.3 (MH^+).

General Procedure for the Preparation of Derivatives 22-24. Example: 1,1'-Biphenyl-4'-(2"-nitrobenzoyl)-4-sulfonamide (22). A mixture of **19** (100 mg, 0.33 mmol), **28** (120 mg, 0.42 mmol), cesium carbonate (140 mg, 0.43 mmol) and Pd(II) acetate (10 mg, 0.033 mmol) in 1-methyl-2-pyrrolidinone (2.9 mL) and water (0.25 mL) was placed into the MW cavity (closed vessel mode, Pmax = 250 PSI). Starting MW irradiation of 100 W was used, the temperature being ramped from 25 °C to 110 °C. Once 110 °C was reached, taking about 2 min, the reaction mixture was held at the same temperature for 15 min while stirring and cooling. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, ethyl acetate:*n*-hexane 7:3 as eluent) to

furnish **22** (64 mg, 51%), mp 202-205 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 7.43 (br s, 2H, disappeared on treatment with D_2O), 7.71 (dd, *J* = 3.7 and 9.8 Hz, 1H), 7.82-7.97 (m, 10H), 8.32 ppm (d, *J* = 7.4 Hz, 1H). IR: ν 1531, 1648, 3265 cm^{-1}. $C_{19}H_{14}N_2O_5S$. MW 382.39. LRMS found (ESI) 383.3 (MH^+).

1,1'-Biphenyl-4'-(3"-nitrobenzoyl)-4-sulfonamide (23). Synthesized as **22** starting from **20** and **28**. Yield 36%, mp >250 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 7.45 (br s, 2H, disappeared on treatment with D_2O), 7.88-8.0 (m, 9H), 8.21 (d, *J* = 7.4 Hz, 1H), 8.48-8.49 (m, 1H), 8.53 ppm (dd, *J* = 2.3 and 8.3 Hz, 1H). IR: ν 1509, 1656, 2963, 3278 cm^{-1}. $C_{19}H_{14}N_2O_5S$. MW 382.39. LRMS found (ESI) 383.2 (MH^+).

1,1'-Biphenyl-4'-(3"-nitrobenzyl)-4-sulfonamide (24). Synthesized as **22** starting from **21** and **28**. It was used as a crude product without further purification. $C_{19}H_{16}N_2O_4S$. MW 368.41. LRMS found (ESI) 369.3 (MH^+).

General Procedure for the Preparation of Derivatives 25-27 and 35. Example: 1,1'-Biphenyl-4'-((2"-nitrophenyl)hydroxymethyl)-4-sulfonamide (25). A mixture of **20** (111 mg, 0.29 mmol) and $NaBH_4$ (11 mg, 0.29 mmol) in tetrahydrofuran (5.5 mL) containing water (0.33 mL) was heated at reflux for 2 h. After cooling, ice and water were added and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a residue that was purified by silica gel column chromatography (chloroform:ethanol 98:2 as eluent) to furnish **25** (96 mg, 86%), mp 155-157 °C (from ethanol). ^{1}H NMR 300 MHz (DMSO-*d*$_6$): δ 6.24 (s, 1H), 6.30 (br s, 1H, disappeared on treatment with D_2O), 7.35-7.37 (m, 4H, disappeared on treatment with D_2O), 7.53 (t, *J* = 6.5 Hz, 1H), 7.67 (d, *J* = 6.3 Hz, 2H), 7.74 (t, *J* = 5.5 Hz, 1H), 7.80-7.90 ppm (m, 6H). IR: ν 2853, 2927, 3259 cm^{-1}. $C_{19}H_{16}N_2O_5S$. MW 384.41. LRMS found (ESI) 385.2 (MH^+).

1,1'-Biphenyl-4'-((3"-nitrophenyl)hydroxymethyl)-4-sulfonamide (26). Synthesized as **25** starting from **23**. Yield 67%, mp 62-65 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 5.95 (d, *J* = 2.9 Hz, 1H), 6.32 (d, *J* = 4.2 Hz, 1H, disappeared on treatment with D_2O), 7.37 (br s, 2H, disappeared on treatment with D_2O), 7.54 (d, *J* = 8.3 Hz, 2H), 7.62 (t, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 8.2 Hz, 2H), 7.81-7.88 (m, 5H), 8.09 (dd, *J* = 2.4 and 8.4 Hz, 1H), 8.29-8.31 ppm (m, 1H). IR: ν 3258 cm^{-1}. $C_{19}H_{16}N_2O_5S$. MW 384.41. LRMS found (ESI) 385.1 (MH^+).

4-Bromophenyl-3'-nitrophenylmethanol (27). Synthesized as **25** starting from **20**. Yield 89%, mp 60-65 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 5.88 (s, 1H), 6.33 (br s, 1H, disappeared on treatment with D_2O), 7.37 (d, *J* = 8.2 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.60 (t, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 8.08 (dd, *J* = 1.1 and 8.0 Hz, 1H), 8.23 ppm (s, 1H). IR: ν 3089, 3411 cm^{-1}. $C_{19}H_{14}BrNO_3$. MW 384.22. LRMS found (ESI) 385.0 (MH^+).

1-(4'-Bromophenyl)-1,3-dihydroisobenzofuran-3-one (35). Synthesized as **25** starting from **33**. Yield 73%, mp 133-136 °C (from ethanol). ^{1}H NMR 400 MHz ($CDCl_3$): δ 6.37 (s, 1H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 7.7 Hz, 1H), 7.52-7.54 (m, 2H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.67 (t, *J* = 8.4 Hz, 1H), 7.98 ppm (d, *J* = 7.8 Hz, 1H). IR: ν 1754 cm^{-1}. $C_{20}H_{13}BrO_2$. MW 365.22. LRMS found (ESI) 366.1 (MH^+).

Ethyl 3-((4'-bromophenyl)hydroxymethyl)benzoate (36). Sodium borohydride (60 mg, 0.4 mmol) was added to a solution of **34** (120 mg, 0.4 mmol) in ethanol (30 mL). The reaction mixture was heated at reflux for 2 h. After cooling, the mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, ethyl acetate:*n*-hexane 1:8 as eluent) to furnish **36** (51 mg, 38%) as an oil. ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 1.3 (t, *J* = 7.0 Hz, 3H), 4.27-4.32 (m, 2H), 5.78 (s, 1H), 6.14 (br s, 1H, disappeared on treatment with D_2O), 7.32 (d, *J* = 8.4 Hz, 2H), 7.43-7.51 (m, 3H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.97 ppm (s, 1H). IR: ν 1697, 2981, 3424 cm^{-1}. $C_{22}H_{19}BrO_3$. MW 411.29. LRMS found (ESI) 412.1 (MH^+).

General Procedure for the Preparation of Derivatives 40-44. Methyl 2"-(1,1'-biphenyl-4-sulfamoyl-4'-ylcarbonyl)benzoate (40). Synthesized as **6** starting from **33** and **28**. Yield 30%, mp 183-186 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 3.61 (s, 3H), 7.43 (br s, 2H, disappeared on treatment with D_2O), 7.52 (d, *J* = 7.5 Hz, 1H), 7.69-7.75 (m, 3H), 7.77-7.81 (m, 1H), 7.87-7.89 (m, 2H), 7.92 (s, 4H), 8.03 ppm (d, *J* = 7.8 Hz, 1H). IR: ν 1669, 1725, 3234 cm^{-1}. $C_{21}H_{17}NO_5$.S MW 395.43. LRMS found (ESI) 396.3 (MH^+).

Methyl 2"-(1,1'-biphenyl-4-sulfamoyl-4'-ylmethyl)benzoate (41). Synthesized as **6** starting from **38** and **28**. Yield 39%, mp 121-124 °C (from ethanol). ^{1}H NMR 300 MHz (DMSO-*d*$_6$): δ 3.77 (s, 3H), 4.34 (s, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.33-7.39 (m, 4H, disappeared on treatment with D_2O), 7.50-7.55 (m, 1H), 7.61 (d, *J* = 8.1 Hz, 2H), 7.79-7.86 ppm (m, 5H). IR: ν 1709, 2923, 3266, 3361 cm^{-1}. $C_{21}H_{19}NO_4$.S MW 381.44. LRMS found (ESI) 382.3 (MH^+).

Methyl 3"-(1,1'-biphenyl-4-sulfamoyl-4'-ylcarbonyl)benzoate (42). Synthesized as **6** starting from **34** and **28**. Yield 40%, mp 91-94 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d6*): δ 3.88 (s, 3H), 7.45 (br s, 2H, disappeared on treatment with D_2O), 7.75 (t, *J* = 7.1 Hz, 1H), 7.87-7.89 (m, 2H), 7.93-7.99 (m, 6H), 8.06 (d, *J* = 8.0 Hz, 1H), 8.25-8.28 ppm (m, 2H). IR: ν 1654, 1717, 3248, 3358 cm^{-1}. $C_{21}H_{17}NO_5$.S MW 395.43. LRMS found (ESI) 396.2 (MH^+).

Ethyl 3"-(1,1'-biphenyl 4-sulfamoyl-4'-ylmethyl)benzoate (43). Synthesized as **6** starting from **39** and **28**. Yield 64%, mp 175-177 °C (from ethanol). ^{1}H NMR 300 MHz (DMSO-*d*$_6$): δ 1.29 (t, *J* = 5.3 Hz, 3H), 4.07 (s, 2H), 4.25-4.31 (m, 2H), 7.35-7.37 (m, 4H, disappeared on treatment with D_2O), 7.43-7.47 (m, 1H), 7.55 (d, *J* = 5.7 Hz, 1H), 7.65 (d, *J* = 6.1 Hz, 2H), 7.78-7.86 ppm (m, 6H). IR: ν 1708, 1717, 3247, 3353 cm^{-1}. $C_{21}H_{19}NO_4$.S MW 381.44. LRMS found (ESI) 382.1 (MH^+).

Ethyl 3"-(1,1'-biphenyl-4-sulfamoyl-4'-ylhydroxymethyl)benzoate (44). Synthesized as **6** starting from **38** and **28**. Yield 69%, mp 140-143 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 1.31 (t, *J* = 7.1 Hz, 3H), 4.28-4.33 (m, 2H), 5.86 (s, 1H), 6.12 (br s, 1H, disappeared on treatment with D_2O), 7.35 (br s, 2H, disappeared on treatment with D_2O), 7.45-7.51 (m, 3H), 7.68 (d, *J* = 8.2 Hz, 3H), 7.81-7.88 (m, 5H), 8.03 ppm (s, 1H). IR: ν, 1717, 3252, 3350 cm^{-1}. $C_{22}H_{21}NO_5S$. MW 411.47. LRMS found (ESI) 412.3 (MH^+).

General Procedure for the Preparation of Derivatives 21 and 39. Example: Ethyl 3-(4'-bromobenzyl)benzoate (39). A mixture of **36** (70 mg, 0.6 mmol) in chloroform (18 mL) was cooled in an ice bath. Trifluoromethanesulfonic acid (220 mg, 0.13 mL, 0.15 mmol) was added over 30 min. After stirring at 25 °C overnight, the mixture was extracted with choloroform. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, ethyl acetate:*n*-hexane 1:5 as eluent) to furnish **39** (30 mg, 68%) as an oil. ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 1.29 (t, *J* = 7.1 Hz, 3H), 4.01 (d, *J* = 6.2 Hz, 2H), 4.26-4.31 (m, 2H), 7.17-7.31 (m, 3H), 7.42-7.52 (m, 3H), 7.78-7.8 ppm (2H). IR: ν 1714, 2981 cm^1. $C_{22}H_{21}NO_5S$. MW 411.47. LRMS found (ESI) 412.3 (MH^+).

1-(4'-Bromobenzyl)-3-nitrobenzene (21). Synthesized as **39** starting from **27**. Yield 65% as an oil. ^{1}H NMR 400 MHz (DMSO-*d*$_6$): δ 4.08 (s, 2H), 7.25 (d, *J* = 8.3 Hz, 2H), 7.49 (d, *J* = 8.3 Hz, 2H), 7.59 (t, *J* = 7.9 Hz, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 8.05-8.10 ppm (m, 2H). IR: ν 1350, 1527, 2927 cm^{-1}. $C_{13}H_{10}BrNO_2$. MW 292.13. LRMS found (ESI) 293.0 (MH^+).

2-(4'-Bromobenzyl)benzoic acid (37). Chlorotrimethylsilane (175 mg, 0.2 mL, 1.6 mmol) was added to a stirred solution of **35** (134 mg, 0.46 mmol) and NaI (241 mg, 1.6 mmol) in dry acetonitrile (4 mL). The reaction mixture was refluxed overnight under Ar stream. After cooling the product was hydrolysed to the corresponding carboxylic acid by adding distilled water (2 mL) while stirring. The reaction mixture was extracted with ethyl acetate and washed

with sodium thiosulfate solution and water to remove iodine and inorganics salts. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, ethyl acetate:*n*-hexane 1:1 as eluent) to furnish **37** (90 mg, 67%), mp 130-133 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-d_6): δ 4.30 (s, 2H), 7.09 (d, J = 8.4 Hz, 2H), 7.27-7.34 (m, 2H), 7.42-7.49 (m, 3H), 7.81 (d, J = 7.6 Hz, 1H), 12.94 ppm (br s, 1H, disappeared on treatment with D_2O). IR: ν 1671, 2192 cm^{-1}. $C_{14}H_{11}BrO_2$. MW 291.14. LRMS found (ESI) 291.9 (MH^+).

General Procedure for the Preparation of Derivatives 33, **34** and **38**. **Example: Methyl 2-(4'-bromobenzoyl)benzoate (31).** A mixture of **31** (190 mg, 0.62 mmol), methanol (2.1 mL) and sulfuric acid 96% (0.21 mL) was refluxed for 12 h. After cooling, the reaction mixture was made alkaline with NaOH and extracted with ethyl acetate. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a residue that was purified by column chromtaoghraphy (silica gel, chloroform:ethanol 95:5 as eluent) to furnish **31** (162 mg, 81%), mp 102-105 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-d_6): δ 3.59 (s, 3H), 7.5 (d, J = 7.5 Hz, 1H), 7.53-7.56 (m, 2H), 7.68-7.8 (m, 4H), 8.01 ppm (d, J = 7.6 Hz, 1H). IR: ν 1666, 1718, 2927 cm^{-1}. $C_{15}H_{11}BrO_3$. MW 319.15. LRMS found (ESI) 320.0 (MH^+).

Methyl 3-(4'-bromobenzoyl)benzoate (34). Synthesized as **31** starting from **32**. Yield 74%, mp 89-92 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-d_6): δ 3.88 (s, 3H), 7.67-7.75 (m, 3H), 7.80 (d, J = 8.4 Hz, 2H), 7.99-8.02 (m, 1H), 8.23-8.25 ppm (m, 2H). IR: ν 1655, 1714, 2919 cm^{-1}. $C_{15}H_{11}BrO_3$. MW 319.15. LRMS found (ESI) 319.9 (MH^+).

Methyl 2-(4'-bromobenzyl)benzoate (36). Synthesized as **33** starting from **37**. Yield 63% as an oil. ^{1}H NMR 400 MHz (DMSO-d_6): δ 3.74 (s, 3H), 4.24 (s, 2H), 7.06 (d, J = 10.6 Hz, 2H), 7.32-7.37 (m, 2H), 7.42 (d, J = 10.4 Hz, 2H), 7.49-7.54 (m, 1H), 7.78 ppm (d, J = 10.6 Hz, 1H). IR: ν 1717, 2841 cm^{-1}. $C_{15}H_{13}BrO_2$. MW 305.17. LRMS found (ESI) 306.1 (MH^+).

General Procedure for the Preparation of 20, 29 and 30. Example: (4'-Bromophenyl)(3-nitrophenyl)methanone (20). To a suspension of anhydrous $AlCl_3$ (3.6 g, 27 mmol) in bromobenzene (21.2 g, 135 mmol) was added dropwise 3-nitrobenzoyl chloride (5.0 g, 27 mmol) in the same solvent (10 mL). The reaction mixture was refluxed for 1.5 h. After cooling, the mixture was quenched on crushed ice/6N HCl and extracted with chloroform. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a residue that was triturated in *n*-hexane:diethyl ether 7:3 to furnish **20** (5.4 g, 67%), mp 98-100 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-d_6): δ 7.72-7.75 (m, 2H), 7.80-7.88 (m, 3H), 8.16 (d, J = 6.4 Hz, 1H), 8.44 (t, J = 1.7 Hz, 1H), 8.5-8.52 ppm (m, 1H). IR: ν 1531, 1649, 3084 cm^{-1}. $C_{13}H_8BrNO_2$. MW 306.11. LRMS found (ESI) 307.1 (MH^+).

(4'-Bromophenyl)(2-methylphenyl)methanone (27). Synthesized as **20** starting from 2-methylbenzoyl chloride. Yield 51% as an oil. ^{1}H NMR 400 MHz (DMSO-d_6): δ 2.22 (s, 3H), 7.30-7.32 (m, 2H), 7.37 (d, J = 7.9 Hz, 1H), 7.44-7.49 (m, 1H), 7.60-7.63 (m, 2H), 7.75-7.77 ppm (m, 2H). IR: ν 1664, 2926 cm^{-1}. $C_{14}H_{11}BrO$. MW 275.14. LRMS found (ESI) 276.0 (MH^+).

(4'-Bromophenyl)(3-methylphenyl)methanone (30). Synthesized as **20** starting from 3-methylbenzoyl chloride. Yield 30%, mp 93-96 °C (from ethanol). ^{1}H NMR 300 MHz (DMSO-d_6): δ 2.38 (s, 3H), 7.41-7.54 (m, 4H), 7.65 (d, J = 8.6 Hz, 2H), 7.77 ppm (d, J = 8.5 Hz, 2H). IR: ν 1649, 2919 cm^{-1}. $C_{14}H_{11}BrO$. MW 275.14. LRMS found (ESI) 276.1 (MH^+).

General Procedure for the Preparation of Derivatives 31 and 32. Example: 2-(4'-Bromobenzoyl)benzoic acid (31). Potassium permanganate (710 mg, 4.5 mmol) was added portionwise to compound **29** (250 mg, 0.9 mmol) in *tert*-butyl alcohol (1.25 mL) and water (2 mL) at 50 °C. The reaction mixture was heated at 80 °C for 5 h. After cooling the reaction mixture was cooled at 0 °C, made acid with 6N HCl and extracted with ethyl acetate. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a

residue that was purified by column chromatoghraphy (silica gel, chloroform:ethanol 95:5 as eluent) to furnish **31** (200 mg, 73%), mp 174-176 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-d_6): δ 7.4 (d, J = 7.3 Hz, 1H), 7.51 (d, J = 8.5 Hz, 2H), 7.62-7.65 (m, 1H), 7.68-7.71 (m, 3H), 7.98 ppm (d, J = 7.8 Hz, 1H). IR: ν 1672, 2556 cm^{-1}. $C_{14}H_9BrO_3$. MW 305.12. LRMS found (ESI) 306.0 (MH^+).

3-(4'-Bromobenzoyl)benzoic acid (30). Synthesized as **31** starting from **25**. It was used as a crude product without further purification. $C_{14}H_9BrO_3$. MW 305.12. LRMS found (ESI) 305.9 (MH^+).

(4'-Bromophenyl)(2-nitrophenyl)methanone (17). 2-Nitrobenzoyl chloride (3.0 g, 16 mmol) was dissolved in 1,2-dichloroethane (6 mL) and bromobenzene (4.0 g, 2.7 mL, 24 mmol) and cooled to 0 °C. Anhydrous iron(III) chloride (2.9 g, 18 mmol) was added by 3 portions over 30 min. The reaction mixture was stirred at 0 °C for 1 h. The mixture was diluted with water and extracted with dichloromethane. The organic layer was washed with brine, dried and filtered. Removal of the solvent gave a residue that was purified by column chromatography (silica gel, ethyl acetate: *n*-hexane = 1:3 as eluent) to furnish **17** (200 mg, 5%), mp 157-159 °C (from ethanol). ^{1}H NMR 400 MHz (DMSO-d_6): δ 7.64 (d, J = 8.6 Hz, 2H), 7.68 (dd, J = 1.3 and 7.5 Hz, 1H), 7.76 (d, J = 8.6 Hz, 2H), 7.84-7.88 (m, 1H), 7.94-7.98 (m, 1H), 8.2944-8.31 ppm (m, 1H). IR: ν 1648, 2923, 2959 cm^{-1}. $C_{13}H_8BrNO_3$. MW 306.11. LRMS found (ESI) 307.1 (MH^+).

CA inhibition assay. The inhibition assay of selected CA isozymes was performed using SX.18V-R Applied Photophysics (Oxford, UK) stopped flow instrument.[16] 10 mM Hepes (pH 7.4) as a buffer, with Phenol Red (at a concentration of 0.2 mM) as an indicator, 0.1 M Na_2SO_4 or $NaClO_4$ (To maintain constant the ionic strength; these anions are not inhibitory in the used concentration), following the CA-catalyzed CO_2 hydration reaction for a period of 5-10 s. Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10mM (in DMSO-water 1:1, v/v) and dilutions up to 0.01 nM done with the assay buffer mentioned above. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were pre-incubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. Triplicate experiments were done for each inhibitor concentration, and the values reported in this paper are the mean of such results. The inhibition constants were obtained by non-linear least squares methods using the Cheng-Prusoff equation, as reported earlier,[17] and represent the mean from at least three different determinations. All CA isozymes used here were recombinant proteins obtained as reported earlier by our group.[18,19]

In silico Evaluation

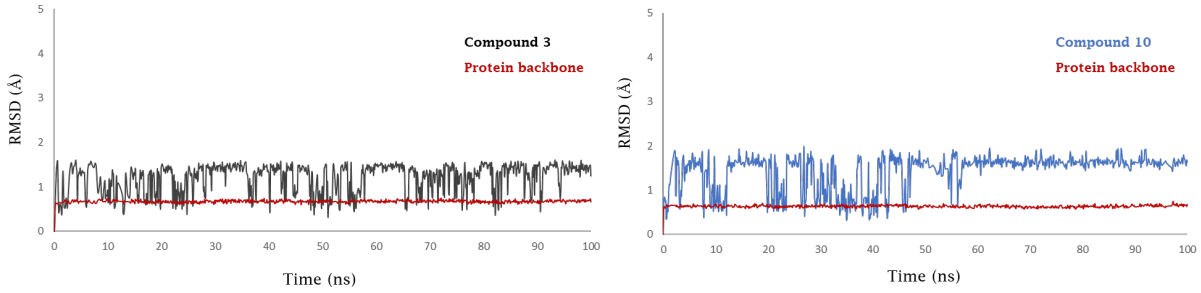

Figure S1. RMSD analysis of the protein backbone and the heavy atoms of **3** (panel A) or **10** (panel B) over the 100 ns MD.

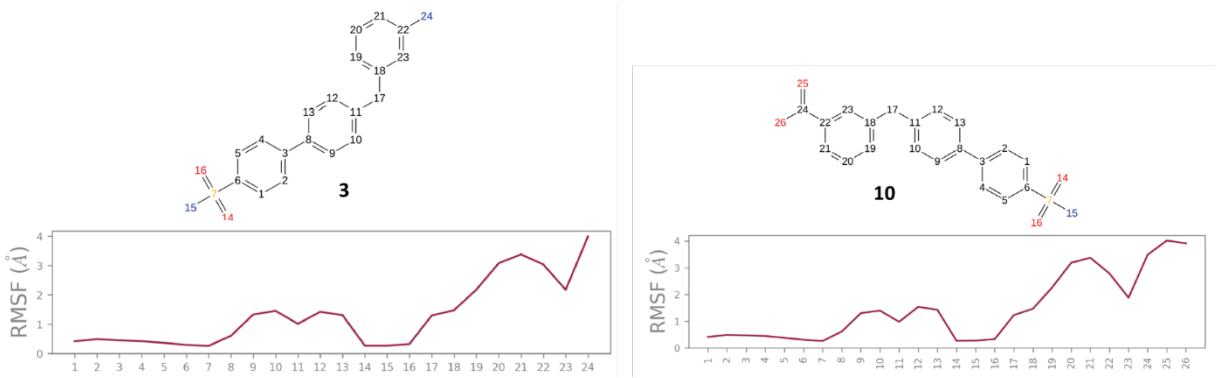

Figure 2S. RMSF analysis of the heavy atoms of compounds **3** and **10** in the MD simulation.

Methods. The crystal structure of hCA II (pdb 5LJT)[20] was prepared using the Protein Preparation Wizard tool implemented in Maestro - Schrödinger suite, assigning bond orders, adding hydrogens, deleting water molecules, and optimizing H-bonding networks.[21] Energy minimization protocol with a root mean square deviation (RMSD) value of 0.30 was applied using an Optimized Potentials for Liquid Simulation (OPLS3e) force field. 3D ligand structures were prepared by Maestro[21b] and evaluated for their ionization states at pH 7.4 ± 0.5 with Epik.[21c] OPLS3e force field in Macromodel [21d] was used for energy minimization for a maximum number of 2500 conjugate gradient iteration and setting a convergence criterion of 0.05 kcal $mol^{-1}Å^{-1}$. The docking grid was centred on the center of mass of the co-crystallized ligands and Glide used with default settings. Ligands were docked with the standard precision mode (SP) of Glide[21e] and the best 5 poses of each molecule retained as output. The best pose for each compound, evaluated in terms of coordination, hydrogen bond interactions and hydrophobic contacts, was refined with Prime[21a] with a VSGB solvation model considering the target flexible within 3Å around the ligand.[22,23]
The best scored binding poses of **3** and **10** to hCA II were submitted to a MD simulation using Desmond[24] and the OPL3e force field. Specifically, the system was solvated in an orthorhombic box using TIP4PEW water molecules, extended 15 Å away from any protein atom. It was neutralized adding a concentration of 0.15 M chlorine and sodium ions. The simulation protocol included a starting relaxation step followed by a final production phase of 50 ns. In particular, the relaxation step comprised the following: (a) a stage of 100 ps at 10 K retaining the harmonic restraints on the solute heavy atoms (force constant of 50.0 kcal mol^{-1} $Å^{-2}$) using the NPT ensemble with Brownian dynamics; (b) a stage of 12 ps at 10 K with harmonic restraints on the solute heavy atoms (force constant of 50.0 kcal mol^{-1} $Å^{-2}$), using the NVT ensemble and Berendsen thermostat; (c) a stage of 12 ps at 10 K and 1 atm, retaining the harmonic restraints and using the NPT ensemble and Berendsen thermostat and barostat; (f) a stage of 12 ps at 300 K and 1 atm, retaining the harmonic restraints and using the NPT ensemble and Berendsen thermostat and barostat; (g) a final 24 ps stage at 300 K and 1 atm without harmonic restraints, using the NPT Berendsen thermostat and barostat. The final production phase of MD was run using a canonical the NPT Berendsen ensemble at temperature 300 K. During the MD simulation, a time step of 2 fs was used while constraining the bond lengths of hydrogen atoms with the M-SHAKE algorithm. The atomic coordinates of the system were saved every 100 ps along the MD trajectory. Protein RMSD, ligand RMSD/RMSF, and occupancy of intermolecular hydrogen bonds and hydrophobic

contacts were investigated along the production phase of the MD simulation with the Simulation Interaction Diagram tools implemented in Maestro.

References of SI

17. Nocentini, A.; Trallori, E.; Singh, S.; Lomelino, C.L.; Bartolucci, G.; Di Cesare Mannelli, L.; Ghelardini, C.; McKenna, R.; Gratteri, P.; Supuran, C.T. 4-Hydroxy-3-nitro-5-ureido-benzenesulfonamides Selectively Target the Tumor-Associated Carbonic Anhydrase Isoforms IX and XII Showing Hypoxia-Enhanced Antiproliferative Profiles. *J. Med. Chem*. **2018**, *61*, 10860-10874.
18. Nocentini, A.; Ceruso, M.; Bua, S.; Lomelino, C.L.; Andring, J.T.; McKenna, R.; Lanzi, C.; Sgambellone, S.; Pecori, R.; Matucci, R.; Filippi, L.; Gratteri, P.; Carta, F.; Masini, E.; Selleri, S.; Supuran, C.T. Discovery of β-Adrenergic Receptors Blocker-Carbonic Anhydrase Inhibitor Hybrids for Multitargeted Antiglaucoma Therapy. *J. Med. Chem.* **2018**, *61*, 5380-5394.
19. Nocentini, A.; Bonardi, A.; Gratteri, P.; Cerra, B.; Gioiello, A.; Supuran, C.T. Steroids interfere with human carbonic anhydrase activity by using alternative binding mechanisms. *J. Enzyme Inhib. Med. Chem.* **2018**, *33*, 1453-1459.
20. Nocentini, A.; Ferraroni, M.; Carta, F.; Ceruso, M.; Gratteri, P.; Lanzi, C.; Masini, E.; Supuran, C.T. Benzenesulfonamides incorporating flexible triazole moieties are highly effective carbonic anhydrase inhibitors: synthesis and kinetic, crystallographic, computational, and intraocular pressure lowering investigations. *J. Med. Chem.* **2016**, *59*, 10692-10704.
21. Schrödinger Suite Release 2019-1, Schrödinger, LLC, New York, NY, 2019: (a) Prime, v.5.5; Maestro v.11.9; (b) Epik, v.4.7; (c) Impact, v.8.2; (d) Macromodel v.12.3. (e) Glide, v.8.2.
22. Nocentini, A.; Gratteri, P.; Supuran, C. T. Phosphorus versus Sulfur: Discovery of Benzenephosphonamidates as Versatile Sulfonamide-Mimic Chemotypes Acting as Carbonic Anhydrase Inhibitors. *Chemistry* **2019**, *25,* 1188-1192.
23. Nocentini, A.; Carta, F.; Tanc, M.; Selleri, S.; Supuran, C. T; Bazzicalupi, C.; Gratteri, P. Deciphering the Mechanism of Human Carbonic Anhydrases Inhibition with Sulfocoumarins: Computational and Experimental Studies. *Chemistry* **2018**, *24*, 7840-7844.
24. Schrödinger Release 2019-2: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, 2019, v.5.7.